K16 motor domain. The crystal structure revealed that the length of the loop L5 was much shorter than that of conventional kinesin. Therefore, the novel conformation of the L5 of K16 may determine the unique enzymatic properties. Therefore, it is expected that photo regulation of ATPase activity of K16 by incorporation of photochromic molecules in to L5. We prepared the K16 mutants that have elongated different length L5. The result of these mutants ATPace activity suggests that the length of L5 is important for rice kinesin k16 ATPase activity.
Subsequently, we have prepared the K16 mutant Q101C C214S and L5 KIF5A H101C C214S that have a single reactive cysteine reside in the L5. Photochromic molecule of azobenzene derivative PAM or spiropyran derivative MA-SP was incorporated into the cysteine residue to induce conformational change of L5 by ultraviolet and visible light irradiation. The ATPase activities of kinesin mutants modified by PAM or MA-SP showed reversible alteration by ultraviolet and visible light irradiations.

649-Pos Board B449
The Neck Linker of Kinesin-1 functions as a Regulator of ATP Hydrolysis Reaction
Xiao Ling, Masahide Kikkawa, Michio Tomishige.
Kinesin-1 is a highly processive motor that moves along microtubule in a hand-over-hand manner. The neck linker that connects two motor domains has been thought to act as a mechanical amplifier that propels the tethered head forward, however, we recently showed that the neck linker docking is not essential for the forward stepping (Isojima et al. this meeting). We hypothesized that the neck linker docking rather functions to activate ATP hydrolysis reaction.
To test this hypothesis, we engineered various monomeric neck linker mutant kinesin and investigated the effect on the ATP hydrolysis reaction. As the neck linker was deleted further from the C-terminus, microtubule-activated ATPase rate was decreased and became almost undetectable when the whole neck linker was removed. The hydrophobic and complementary shaped side-chain of Ile325 is critical for promoting ATP hydrolysis reaction because substitution of this residue alone into glycine caused dramatic decrease in the ATPase rate. Single molecule fluorescent imaging showed that the Ile325G mutant monomer stably bound to the microtubule even in the presence of saturating ATP. A high-resolution cryo-electron microscopic study of the Ile325G mutant in complex with microtubule showed that the kinesin core is rotated clockwise direction even in the presence of saturating AMPPNP, which is similar to the conformation of wild type in the no-nucleotide state.
These results suggest that Ile325 is essential for promoting ATP hydrolysis reaction by stabilizing counter clockwise rotation of kinesin motor domain upon ATP binding. This mechanism can also explain the front head gating mechanism for dimeric kinesin: the neck linker of the leading head is pulled backward so that the head cannot proceed ATP hydrolysis cycle until the trailing head detaches from microtubule.

650-Pos Board B450
Differences in Processivity between Kinesin Motor Families 1, 2, 3, 5 and 7 Result from Diversity in the Length of their Neck-Linker Domains
Shankar Shastry, William O. Hancock.
Understanding the mechanical and biochemical determinates of kinesin processivity is important for defining the chemomechanical mechanism of kinesin and for uncovering how different kinesins are tuned for specific cellular functions. Because the neck-linker domain is a key mechanical element that underlies coordinated stepping, we previously investigated the effects of neck-linker length, charge and structure in the processivity of Kinesin-1 and Kinesin-2 motors. When the 14 amino acid long neck-linker of Kinesin-1 was extended, processivity was diminished, and conversely, shortening the 17 amino acid long Kinesin-2 neck-linker enhanced processivity. From stochastic simulations of the hydrolysis cycle, these effects can best be explained by a combination of slower strain-dependent detachment of rear head and a reduced strain-dependent inhibition of ATP binding. To test the degree to which the processivity of other N-terminal kinesins is determined by their neck-linker domains, we investigated motor domains of Kinesin-3 (CeUnc104, NL=17 residues), Kinesin-5 (XlKSP, NL=18 residues) and Kinesin-7 (XlCENP-E, NL=18 residues), fused to the Kinesin-1 coiled-coil and GFP. With native neck-linkers, run lengths were 0.6 μm for Kinesin-3, 0.9 μm for Kinesin-7 and below our detection limit for Kinesin-5. Surprisingly, when their neck-linkers were shortened to 14 amino acids, Kinesin-3 and Kinesin-5 run lengths matched Kinesin-1, as did Kinesin-7 after the end of α-6 was changed to match Kinesin-1 sequence. This convergence of processivity is observed even though the speeds of these motors varied over a 25-fold range. These results suggest that diverse N-terminal kinesins are inherently processive to the same degree, and their wild-type behavior results from differences in the length and sequence of their neck-linker domains.

651-Pos Board B451
Casein Kinase 2 Reverses Tail-Independent Inhibition of Kinesin-1
Jing Xu, Zhanyong Shu, Preetha Anand, Babu J.N. Reddy, Silvia Cermelli, Thomas Whisenant, Stephen J. King, Lee Bardwell, Lan Huang, Steven P. Gross.
Kinesin-1 is a plus-end microtubule-based molecular motor, and defects in kinesin transport are linked to diseases including neurodegeneration. Kinesin can auto-inhibit via a direct head-tail interaction, but is believed to be active otherwise. In contrast, this study uncovers a fast but reversible inhibition distinct from the canonical auto-inhibition pathway. The majority of the initially active kinesin (full-length or tail-less) loses its ability to bind/interact with microtubule, and Casein Kinase 2 (CK2) reverses this inactivation (up to 4-fold) without altering kinesin's single motor properties. Motor phosphorylation is not required for this CK2 -mediated kinesin activation. In cultured mammalian cells, knockdown of CK2 level, but not kinase activity, was sufficient to decrease the force required to stall lipid droplet transport, consistent with a reduction in the number of active motors. We propose that CK2 forms a positive regulating complex with the motor. This study provides the first direct evidence of a protein kinase positively regulating kinesin-transport, and uncovers a pathway whereby inactive cargo-bound kinesin can be activated.

652-Pos Board B452
Limping Factors for Motor Proteins
Yunxin Zhang, **Michael E. Fisher**.
Conventional kinesin is a two-headed motor protein that walks ''hand-over-hand'' along microtubules, with the two heads (or motor domains) moving alternatively. *In vitro* single-molecule measurements by Asbury, Fehr and Block [Science **302** (2003) 2130; also Fehr *et al*, Biophys. J. **97** (2009) 1663] find that the stepping motion of kinesin embodies a *limp*, i.e., alternate steps have different mean dwell times. We report an analytical study of the statistics of the observed *limping factor*, L, defined as the ratio of average dwell times for the slow phase to those for the fast phase. Employing basic models we find that, owing to the finite run lengths of experimental measurements, the observed limping factor is always significantly larger than the ''true'' or *intrinsiclimping factor*, L_0. Essentially, for large L_0 the average observed limping factor, $\langle L_n \rangle$,for runs of n odd steps interlaced with n even steps is at least $n/(n\text{-}1)$ times larger than the intrinsic factor, L_0. Thus, if L_0 = 1.0, so that the walk is *limp-free*, the observed mean values for runs of $2n$ = 8, 10, and 12 steps would be $\langle L_n \rangle$ = 2.06, 1.87, and 1.74, respectively. Moreover, the corresponding standard deviations are D L_n= 1.41, 1.04, and 0.83. To improve estimates of L_0 on the basis of observed values, the analysis has been extended (a) to allow for runs of length at least $2k$ steps terminated stochastically by detachment from the track and (b) to utilize information, obtained experimentally or hypothesized, regarding the dwell-time distributions. Reliable estimates of intrinsic limping factors should be useful in untangling the details of asymmetric hand-over-hand stepping in processive motor proteins.

653-Pos Board B453
Structural Basis for the Coordinated Processive Movement of Kinesin-1 studied by Structural and Single Molecule Analyses
Tsukasa Makino, Teppei Mori, Ryo Kanada, Ken-ichi Miyazono, Shoji Takada, Masaru Tanokura, Michio Tomishige.
Kinesin-1 is a dimeric motor protein that moves along microtubules in a hand-over-hand manner. Recent single molecule studies uncovered how ATP hydrolysis cycle is couple to the conformational changes of kinesin dimer, although structural basis for the coordinated walking mechanism still remained unknown. At the last annual meeting, we reported the first crystal structure of nucleotide-free kinesin-1 and presented a model to explain mechanochemical coupling of kinesin motor domain. Here, we modeled atomic-detailed kinesin dimer structures on microtubule at various nucleotide states, based on docking of the crystal structures to high-resolution cryo-EM density maps (Sindelar et al. 2010) and refinement of the neck-linker structures using MD simulations. Based on the analysis of these dimer structures, we propose a model to explain the coordinated motility of kinesin-1: 1) In the two-head bound state, ATP binding to the leading head is prohibited because the neck-linker will be stretched out, explaining the front-head-gating mechanism. 2) In the one-head-bound state, ADP release from the tethered head is prohibited at the rear binding site and only permitted at the forward binding site because the neck-linker will be again stretched out, which explains the forward stepping mechanism. These models provide a testable prediction that if the neck-linker is artificially extended, kinesin can adopt these off-pathway conformations because the tension is now relaxed, and single molecule FRET analysis of neck linker extension mutants provided experimental support. These results suggest that two kinesin motor domain can coordinate to move hand-over-hand because